

IN THE SPECIFICATION:

Please amend the paragraph beginning at page 14, line 17 as follows:

~~Figure 1 shows~~Figures 1(a)-1(e), joined by the match lines A-A through D-D,
show the nucleic acid sequence (SEQ ID NO. 2) of the pNA-PI-2 insert and the
corresponding amino acid sequence (SEQ ID NO. 3) of the *N. alata* PI protein. The
amino acid sequence is numbered beginning with 1 for the first amino acid of the mature
protein. The signal sequence is encoded by nucleotides 1 to 97 and the amino acid
residues have been assigned negative numbers. The reactive site residues of the inhibitor
are boxed. The *N. alata* PI sequence contains six similar domains (domain 1, residues 1
to 58, domain 2, residues 59-116, domain 3, residues 117-174, domain 4 residues 175-
232, domain 5, residues 233-290 and domain 6, residues 291-343).

Please amend the paragraph beginning at page 15, line 29 as follows:

~~Figure 5 is a graphic representation~~Figures 5a-5b are graphic representations of PI
activity in various organs of *N. alata*. Buffer soluble extracts from various organs were
tested for their ability to inhibit trypsin and chymotrypsin. Stigma and sepal extracts
were the most effective inhibitors of both trypsin (Aa) and chymotrypsin (Bb).

Please amend the paragraph beginning at page 16, line 1 as follows:

~~Figure 1 depicts~~Figures 6a-6c depict the steps of the purification of PI from *N.*
alata stigmas.

Please amend the paragraph beginning at page 16, line 17 as follows:

~~Figure 7 is a graphical representation~~Figures 7a-7c are graphic representations
showing hydropathy plots of the PI proteins encoded by the NA-PI-2 clone from *N. alata*
and the potato and tomato PI II cDNAs. Values above the line denote hydrophobic

regions and values below the line denote hydrophilic regions. The putative signal peptides are shaded. The hydrophobicity profile was generated using the predictive rules of Kyte and Doolittle (1982) and a span of 9 consecutive amino acids.

Please amend the paragraph beginning at page 17, line 7 as follows:

~~Figure 8 shows~~ Figures 8a-8c show an immunoblot analysis of the PI protein in stigmas of developing flowers.



Please amend the paragraph beginning at page 17, line 23 as follows:

~~Figure 9 shows~~ Figures 9a-9b show the separation and identification of the 6kD proteinase inhibitor species from *N.alata* stigmas.

Aa. Separation of the 6kD PIs by reversed phase HPLC chromatography.

Four major peaks were obtained with retention times of about 15.5min(peak1), 20.5 min (peak2), 22.5min(peak3), 24min(peak4). The peptides in each peak have been identified by a combination of N-terminal analysis and mass spectrometry. ~~See B for description of C1 and T1-T4.~~

Please amend the paragraph beginning at page 18, line 1 as follows:

~~B.~~ The five homologous peptides produced from the PI precursor protein: C1, chymotrypsin inhibitor, T1-T4 trypsin inhibitors. The solid bars represent the reactive sites of the inhibitors. The precursor protein is drawn minus the signal sequence.  region of the six repeated domains (amino acids 1-343, Fig. 1)..  non-repeated sequence (amino acids 344-368, Fig. 1). The arrows point to the processing sites in the precursor protein.

Please amend the paragraph beginning at page 18, line 9 as follows:

€b. The amino acid sequence of C1 (SEQ ID NO: 5) and T1-T4 (SEQ ID NOS: 6-9, respectively) predicted from the cDNA clone and confirmed by N-terminal sequencing of the purified peptides. The amino acid at the carboxy-terminus of each peptide was obtained by accurate mass determination using an electro-spray mass spectrometer. The C1 and T1 inhibitors differ by five amino acids (bold). Two of these amino acids are located at the reactive site (underlined) and the other two to three reside at the carboxy-terminus. Peptides T2-T4 have changes in three amino acids (boxed) that are conserved between C1 and T1. Peptides T2 and T3 are identical to each other. Mass spectrometry was used to demonstrate that other forms of C1 and T1-T4 occur due to non-precise trimming at the N- and C-termini. That is, some forms are missing residue 1 residue 53 and others are missing both residue 1 and 53 (see Table 2).

Please amend the paragraph beginning at page 18, line 21 as follows:

Figure 10 shows the amino-acid sequence (SEQ ID NO: 16) around the processing sites in the precursor PI protein.

Please amend the paragraph beginning at page 18, line 28 as follows:

~~Figure 11 shows~~ Figures 11a-11b show the PI precursor produced in a baculovirus expression system and the products obtained after digestion of the affinity purified PI precursor by the endoprotease Asp-N.

Please amend the paragraph beginning at page 19, line 1 as follows:

Aa. The PI precursor produced by the recombinant baculovirus. Immunoblot containing affinity and HPLC purified PI precursor from *N.alata* stigmas at the green bud stage of development (lane 1) and affinity purified PI precursor produced

by the recombinant baculovirus (lane 2). Proteins were fractionated by electrophoresis on a 15% w/v SDS-polyacrylamide gel prior to electrophoretic transfer to nitrocellulose. The blot was incubated with the antibody raised in rabbits to the 6kD PI species from stigmas. The recombinant virus produced an immunoreactive protein of 42kD that is the same size as the PI precursor protein produced by stigmas (arrowed).

Please amend the paragraph beginning at page 19, line 12 as follows:

Bb. Cleavage of the PI precursor by endoproteinase Asp-N.
15% SDS-polyacrylamide gel stained with silver containing: 1, PI precursor, produced by baculovirus, incubated without enzyme. 2, enzyme incubated without precursor. 6kD, PI peptides of about 6kD purified from *N.alata* stigmas. 1m, 5m, 30m, reaction products produced after 1, 5 and 30 minutes of incubation. 2h and 24h, reaction products after 2 and 24h of incubation. Peptides of about 6-7kD were detected within one minute of incubation of the precursor with the enzyme. After 24h only peptides of 6-7kD were detected. The bands smaller than 42kD in track 1 are due to truncated forms of the precursor produced by premature termination of translation in the baculovirus expression system.

Please amend the paragraph beginning at page 20, line 1 as follows:

~~Figure 13 shows~~Figures 13a-13b show a comparison of the trypsin and chymotrypsin inhibition activity of the PI precursor, PI peptides from stigmas and in vitro produced PI peptides from the PI precursor.